Acta Pædiatrica ISSN 0803–5253

REGULAR ARTICLE

Circulating leptin levels and bone mineral density in children with biliary atresia

Sittisak Honsawek[1], Tawatchai Chaiwatanarat[2], Voranush Chongsrisawat[3], Nutchanart Thawornsuk[3], Paisarn Vejchapipat[4], Yong Poovorawan (Yong.P@chula.ac.th)[3]

1.Department of Biochemistry, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand
2.Department of Radiology, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand
3.Department of Pediatrics and Center of Excellence in Viral Hepatitis Research, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand
4.Department of Surgery, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand

Keywords
Biliary atresia, Bone mineral density, C-terminal telopeptide of type I collagen, Leptin, Osteocalcin

Correspondence
Prof. Yong Poovorawan, M.D., Center of Excellence in Viral Hepatitis Research, Department of Pediatrics, Faculty of Medicine, Chulalongkorn University and Hospital, Rama IV road, Patumwan, Bangkok, Thailand 10330.
Tel.: +662-256-4909 | Fax: +662-256-4929 | Email: Yong.P@chula.ac.th

Received
23 July 2007; revised 18 September 2007; accepted 23 October 2007.

DOI:10.1111/j.1651-2227.2007.00596.x

Abstract

Aim: To investigate circulating leptin levels in biliary atresia (BA) patients and the association of leptin with bone mineral density (BMD) and the severity of BA.

Methods: We have examined 50 patients with BA and 15 matched healthy controls. Serum leptin, osteocalcin and C-terminal telopeptide of type I collagen (CTX) levels were measured by sandwich enzyme-linked immunosorbent assay (ELISA). BMD of the lumbar spine was measured by dual energy X-ray absorptiometry.

Results: Serum leptin levels of BA patients were lower than those of healthy controls (2.7 ± 0.3 vs. 7.1 ± 1.7 ng/mL, $p = 0.0001$). Among the BA patients, serum leptin levels were significantly lower in patients with jaundice than patients without jaundice (1.7 ± 0.2 vs. 3.4 ± 0.4 ng/mL, $p = 0.001$). BMD of BA patients was correlated ($p < 0.001$) with leptin levels, age and BMI ($r = 0.55$, $r = 0.75$ and $r = 0.58$, respectively). The serum CTX levels were significantly higher in jaundice patients compared with jaundice-free patients and the healthy controls (0.6 ± 0.2 vs. 0.2 ± 0.1 ng/mL, $p = 0.01$), whereas the serum osteocalcin levels in BA patients were not different from those in the controls.

Conclusion: Circulating leptin levels are correlated with BMD and the presence of jaundice in BA, suggesting that the leptin may play a physiological role in maintaining bone mass of BA patients with jaundice.

INTRODUCTION

Biliary atresia (BA) is a congenital obstructive cholangiopathy that causes cirrhosis and end-stage liver disease in children. It is characterized by obliteration of the extrahepatic bile duct leading to intrahepatic cholestasis, widening of the portal tracts, proliferation of bile ductules and progression to hepatic fibrosis (1). Except for liver transplantation, Kasai operation or hepatic portoenterostomy constitutes the only definitive surgical treatment for infants with BA (2). Hepatic osteodystrophy is a well-recognized complication of chronic liver disease including BA (3). The mechanisms leading to hepatic osteodystrophy are still not entirely clear. However, factors such as malabsorption of vitamin D, vitamin K or minerals, hormone deficiencies, decreased physical activity and insufficient exposure to sunlight have been postulated (4,5). Children with BA who have progressive injury of the intrahepatic bile ducts with subsequent cholestasis are at risk for vitamin D deficiency resulting from reduced presence of bile acids in the duodenum. Additional factors related to bone disease include decreased intestinal absorption of calcium and phosphate attributable to fat malabsorption. Hyperbilirubinemia or other substances present in serum may impair osteoblast proliferative capacity and play a role in bone disease associated with cholestatic jaundice (6).

Leptin, a 16-kDa peptide product of the obesity (*ob*) gene, is primarily secreted in mature adipose tissue and in other tissue, including bone marrow adipocytes (7). It targets the central nervous system, particularly the hypothalamus, affecting food intake. The primary effect of leptin appears to be mediated by leptin receptors expressed mainly in the hypothalamus (8). Mutations of the *ob* gene leading to leptin deficiency are the cause of obesity in *ob/ob* mice (9). Experimental studies have shown that the leptin is involved in the regulation of food intake and body composition via a central feedback mechanism (8,9).

Although leptin plays an important role in appetite control, fat metabolism, energy expenditure and body weight regulation, it is also responsible for a variety of physiological processes, including hematopoiesis, angiogenesis, immune function and bone metabolism (10). With leptin levels produced from fat stores influencing bone cell activity, one would expect to observe correlations between circulating leptin levels, anthropometric parameters and bone mineral density (BMD). We hypothesize that serum leptin would be higher in BA patients than in controls. Serum CTX is a validated marker of bone resorption (11) and serum osteocalcin has advantage over other markers of being specific for bone formation (12). Therefore, the current study has been conducted to investigate relationships of serum leptin levels with

BMD and with biochemical markers of bone metabolism in BA patients.

PATIENTS AND METHODS

Patients

Fifty BA patients were recruited into the study during their annual routine follow-up between July 2005 and March 2006. The study group included 20 boys (40%) and 30 girls (60%) with a mean age of 7.3 ± 0.6 years. The control group comprised 15 healthy children, age and gender matched with a mean age of 8.0 ± 1.1 years (7 boys and 8 girls) and chosen among those participating in an evaluation of hepatitis B vaccine during the same period. None of the BA patients in this study had undergone liver transplantation or exhibited signs and symptoms of fever or ascending cholangitis at the time of blood sampling. None of the study patients had previously received exogenous steroid treatment. The serum specimens were collected and stored at –80°C until assayed.

In order to compare the outcome among BA patients, they were divided into two groups based on the status of jaundice: patients with jaundice (TB ≥ 2.0 mg/dL, n = 20) and patients without jaundice (TB < 2.0 mg/dL, n = 30). Portal hypertension (PH) was validated by the presence of ascites and/or oesophageal varices demonstrated by endoscopy. Twenty-two patients had no PH, but the remaining 28 suffered with PH.

The study was approved by the Ethics Committee on Human Research of the Faculty of Medicine, Chulalongkorn University. All parents of children with BA and of the healthy controls were informed of the study's objectives, and written informed consent was obtained from the parents prior to the children entering the study.

Laboratory methods

Serum leptin concentrations were measured from venous fasting blood samples stored at –80°C using a commercially available leptin enzyme-linked immunosorbent assay (ELISA) kit (BioVendor Laboratory Medicine, Inc., Brno, Czech Republic). According to the manufacturer's instructions, the detection limit of this assay was 0.5 ng/mL. At leptin concentrations of 3.5–25.6 ng/mL, the intraassay and interassay coefficients of variation (CV) were 3.0–7.5% and 3.2–9.2%, respectively. Serum osteocalcin as a marker of bone formation was measured by the osteocalcin ELISA kit (Nordic Bioscience Diagnostics, Herlev, Denmark). Intraassay and interassay CV values were 2.6% and 4.7%, respectively, with the detection limit at 0.5 ng/mL. Serum C-terminal telopeptide of type I collagen (CTX) as a marker of bone resorption was detected by the ELISA CrossLaps kit (Nordic Bioscience Diagnostics). Intraassay and interassay CV values were 5.2% and 6.7%, respectively, and the detection limit was 0.01 ng/mL. In addition, liver function tests including serum albumin, total bilirubin (TB), direct bilirubin (DB), aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (AP) and gamma glutamyl transpeptidase (GGT) were measured by the central laboratory using the automated Hitachi 912 test system.

Anthropometry

With the patients wearing light indoor clothes and no shoes, body weight was measured to the nearest 0.1 kg, and height to the nearest 0.5 cm. Body mass index (BMI) was calculated as the weight in kg per height in m^2.

Bone density measurements

All children underwent BMD measurements of the lumbar spine (anteroposterior lumbar vertebrae L1–L4) with dual-energy X-ray absorptiometry (DEXA) using Hologic QDR 2000 (Hologic, Inc., Waltham, MA, USA). The results obtained from comparisons of BMD with age-matched norms were expressed as Z-scores of BMD. Control data were obtained from Caucasian children provided by the Hologic 2000 software. No significant differences in spine BMD between Asian and Caucasian children has been documented (13). According to WHO guidelines, osteoporosis was defined as a spinal BMD equal to or exceeding 2.5 standard deviations (SD) below the average values obtained in an age and gender matched group. Osteopenia was defined as a BMD below 2.5 SD but above 1 SD under the average values. Normal BMD was defined as a spinal BMD equal to or below 1 SD under the average values. BMD was expressed as g/cm^2.

Statistical analysis

All values are expressed as mean ± SEM. Statistical analysis was performed using SPSS software. Comparisons between the groups were performed using Student's *t*-test for unpaired data. The correlation among numerical data was analysed by the Pearson correlation coefficient (r), and multivariate analysis using stepwise regression was performed to identify independent parameters. A p-value <0.05 was considered to indicate statistical significance.

RESULTS

The demographic data, liver function tests, markers of bone metabolism and serum leptin levels of BA patients without jaundice compared to BA patients with persistent jaundice are illustrated in Table 1. Fifty BA patients and 15 healthy controls were included in this study. There were no significant differences in age (7.3 ± 0.6 vs. 8.0 ± 1.1 years) and gender (male:female, 20:30 vs. 7:8) between the BA patients and the controls. All BA patients had undergone Kasai portoenterostomy. There were 30 BA patients without jaundice and 20 BA patients with persistent jaundice.

As shown in Figure 1, serum leptin levels of BA patients were significantly lower than those of the healthy controls (2.7 ± 0.3 vs. 7.1 ± 1.7 ng/mL, p = 0.0001). Among the BA patients, serum leptin levels were significantly decreased in patients with jaundice compared with patients without jaundice (1.7 ± 0.2 vs. 3.4 ± 0.4 ng/mL, p = 0.001) and healthy controls (p = 0.0001). Moreover, non-jaundice BA patients had lower serum leptin levels compared with the controls

Table 1 Demographic data, bone metabolism markers and serum leptin levels of biliary atresia patients based on status of jaundice

	Total	No jaundice	Persistent jaundice	p-value
No. of patients	50	30	20	
Age (years)	7.3 ± 0.6	8.3 ± 0.7	5.8 ± 0.8	0.01
Male:Female	20:30	10:20	10:10	NS
Weight (kg)	24.8 ± 1.5	28.6 ± 2.0	19.2 ± 1.4	0.0005
Height (cm)	118.4 ± 3.0	125.8 ± 4.0	107.3 ± 3.5	0.0005
BMI (kg/m^2)	16.7 ± 0.3	17.2 ± 0.4	15.9 ± 0.4	0.001
Total bilirubin (mg/dL)	4.5 ± 1.0	0.9 ± 0.1	9.8 ± 1.9	0.0005
Direct bilirubin (mg/dL)	3.0 ± 0.8	0.3 ± 0.1	7.0 ± 1.5	0.0005
AST (U/L)	151.9 ± 17.5	90.9 ± 14.0	243.4 ± 28.2	0.0005
ALT (U/L)	128.0 ± 16.4	95.9 ± 19.8	176.2 ± 25.0	0.01
Spine BMD Z-score	−1.3 ± 0.2	−0.7 ± 0.2	−2.3 ± 0.3	0.0005
Osteocalcin (ng/mL)	19.1 ± 2.8	18.0 ± 3.7	20.9 ± 4.0	NS
CTX (ng/mL)	0.4 ± 0.1	0.2 ± 0.1	0.6 ± 0.2	0.01
Leptin (ng/mL)	2.7 ± 0.3	3.4 ± 0.4	1.7 ± 0.2	0.001

The data are expressed as mean ± SEM.
NS = not significant.

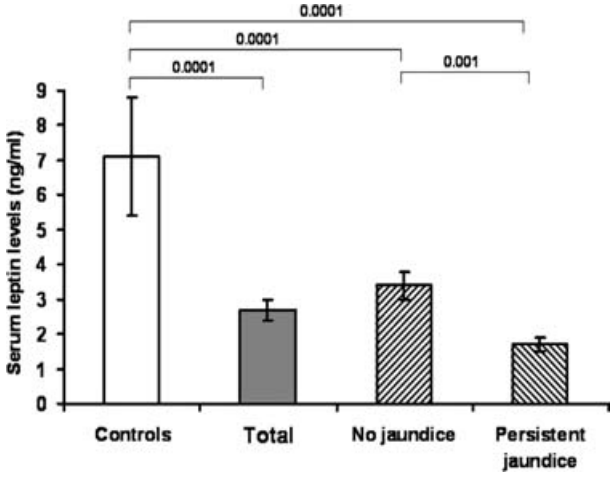

Figure 1 Comparison between serum leptin levels in biliary atresia patients based on total bilirubin and controls. The data are expressed as mean ± SEM.

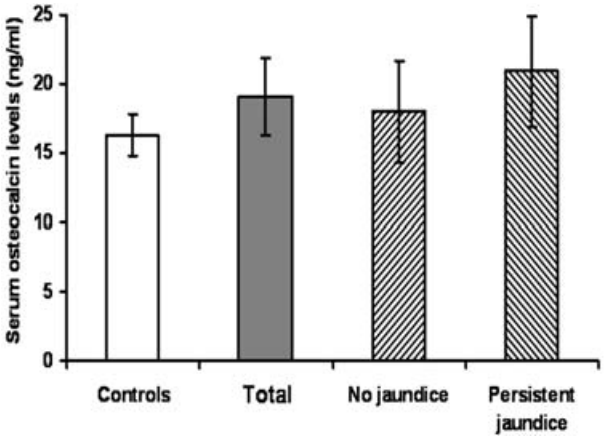

Figure 2 Comparison between serum osteocalcin levels between biliary atresia patients based on total bilirubin and controls. The data are expressed as mean ± SEM.

(p = 0.0001). Further studies revealed that serum leptin levels were significantly reduced in the BA patients with PH (n = 28) compared with those without PH (n = 22) (1.9 ± 0.2 vs. 3.8 ± 0.5 ng/mL, p = 0.001).

BMD of BA patients with persistent jaundice was significantly lower than that in patients without jaundice (p = 0.0005) (Table 1). BMD Z-scores were within the osteopenic range (–2.5 < Z-score < –1.0) in 14 (28%) and within the osteoporotic range (Z-score ≤ –2.5) in 14 (28%) patients. BMD of BA patients was highly correlated (p < 0.001) with circulating leptin levels (r = 0.55), age (r = 0.75) and BMI (r = 0.58).

In order to investigate the underlying mechanism of decreased BMD in patients with BA, we measured the serum levels of osteocalcin and CTX, which are markers of bone turnover. Although the mean serum levels of osteocalcin in patients with BA were higher than those in healthy controls, the difference was not statistically significant (19.1 ± 2.8 vs. 16.3 ± 1.5 ng/mL, p = 0.2) (Fig. 2). There was no significant difference in serum osteocalcin levels between the jaundice group, jaundice-free group and the controls (20.9 ± 4.0, 18.0 ± 3.7 and 16.3 ± 1.5 ng/mL, respectively, p = 0.3).

The mean serum CTX levels in the healthy controls were 0.2 ± 0.1 ng/mL, whereas serum CTX levels in BA children were 0.4 ± 0.1 ng/mL as shown in Figure 3. Thus, the serum CTX values were significantly increased in the BA patients in relation to those of the controls (p = 0.02). In the BA patients, serum CTX levels in the jaundice group were significantly higher than those in the jaundice-free group (0.6 ± 0.2 vs. 0.2 ± 0.1 ng/mL, p = 0.01) and the normal controls (p = 0.01) (Fig. 3).

In multivariate analysis, we assessed the association of clinical outcome as a dependent variable with leptin and CTX as independent variables. Serum leptin levels had a negative association with the severity of BA (p = 0.01) and

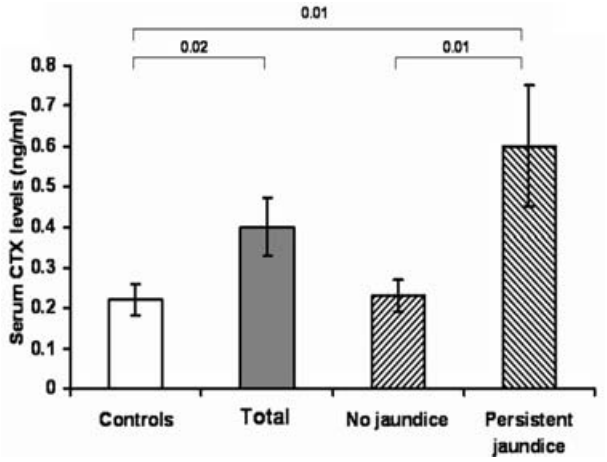

Figure 3 Comparison between serum CTX levels between biliary atresia patients based on total bilirubin and controls. The data are expressed as mean ± SEM.

serum CTX levels were positively associated with the severity of BA ($p = 0.05$).

DISCUSSION

Biliary atresia constitutes one of the most common causes of neonatal obstructive jaundice and malabsorption of nutrients presents a significant problem (14). There are well-documented findings of osteopenia evidenced by cortical thinning and trabecular bone loss occurring in childhood cholestatic liver disease. Several lines of evidence have shown osteodystrophy associated with severe chronic liver disease despite the administration of vitamin and mineral supplements. A previous study has shown that the bone mineral content of hepatic osteodystrophy patients did not improve despite successfully normalizing serum 25-OH vitamin D levels by enhancing vitamin D absorption from the gastrointestinal tract (4). Furthermore, Chongsrisawat et al. have demonstrated that osteoporosis was diagnosed in up to 80% of a group of jaundiced BA patients in comparison with only 13.6% in a nonjaundiced group (15). The severity of jaundice in BA patients is directly correlated to the rate of osteoporosis. BA patients with severe cholestasis are at high risk of bone fractures despite the administration of essential vitamins or minerals.

Recently, role of circulating leptin has been documented in children with immune thrombocytopenic purpura (16,17), celiac disease (18), cystic fibrosis (19) and chronic liver diseases (20–23). However, the role of leptin and bone metabolism in BA patients has not been fully elucidated. To the best of our knowledge, no study dealing with leptin concentrations and BMD in BA patients has been reported in the literature. In this study, we have examined the circulating levels of leptin in BA children and determined the relationship between leptin levels, anthropometric parameters and BMD of the BA patients.

Because bone metabolism dramatically changes in BA and chronic inflammation is the hallmark of the disease, we studied serum levels of leptin in BA patients in comparison with healthy controls. Interestingly, the results showed that serum leptin levels were significantly decreased compared with those of the controls. Decreased serum leptin levels have been demonstrated in chronic viral hepatitis and post-hepatitis liver cirrhosis (24,25). Testa et al. have shown significantly lower serum leptin levels in patients with hepatitis B and C virus infection (24). Greco et al. have demonstrated a direct correlation between severity of liver damage and decline in leptin levels (25). Furthermore, Roberts et al. previously reported that serum leptin was significantly lower in children with end-stage liver disease than in controls and following orthotopic liver transplantation serum leptin was significantly decreased (26). In contrast with these observations, recent studies have indicated that serum leptin levels were increased in patients with liver cirrhosis (27–29). Short-term corticosteroid administration could induce an elevation in serum leptin (30). Although corticosteroid-treated infants showed a significant increase in circulating leptin, this effect was transient and reversible after corticosteroid withdrawal (30). The discrepant findings may reflect differences in the studied sample characteristics (age, sex, race, disease, condition), the size of populations or the statistical analyses. In addition, the present study showed that BMD of BA patients was closely related to leptin levels, age and BMI. Although the exact mechanism culminating in low serum leptin levels has not been elucidated, nutritional and metabolic abnormalities including malnutrition in BA may, at least partly, explain low leptin levels. Additional studies will be required to investigate causes of decreased serum leptin levels in BA patients.

Remodelling, consisting of repeated cycles of resorption and formation, is a lifelong process responsible for the maintenance of bone mass. The remodelling process plays a critical role during the growth phases in metabolic bone disease (31–33). Because biochemical markers of bone turnover are essential for assessing osteoblastic and osteoclastic functions, in this study we have measured the serum osteocalcin and C-terminal cross-linking telopeptide of type I collagen (CTX) levels. Osteocalcin is a non-collagenous protein secreted by osteoblasts during bone matrix mineralization and has been widely accepted as a marker for osteoblastic activity and bone formation, whereas serum CTX, as a collagen-degradation product, is a marker of bone resorption. The insignificant statistical difference in the osteocalcin levels between the BA patients and controls indicates normal osteoblastic function in BA. However, serum CTX levels were significantly increased in patients with BA, which reflects increased bone resorption in BA patients, particularly in those with jaundice.

This study has had various limitations. First, the sample size was not large enough to arrive at definitive conclusions. Secondly, we examined only those subjects who attended Chulalongkorn Memorial Hospital, a tertiary care centre, for evaluation or treatment of BA. Thirdly, circulating leptin levels and BMD values are closely related. Hence, multiple regression analysis performed on these variables may be problematic, and we should interpret the obtained results carefully.

In summary, the present study has detected a significant decrease in circulating leptin levels in children with BA, and in particular in BA patients with persistent jaundice compared to BA patients without jaundice or healthy controls. Our statistical analysis has revealed that the circulating leptin levels were significantly associated with BMD values as well as presence of jaundice in BA. Hence, our results support the suggestion that circulating leptin may exert a physiological role in maintaining bone mass of BA patients with persistent jaundice. Further studies will be required to determine whether leptin plays a regulatory role in bone formation and resorption. Elucidation of such mechanisms may result in a novel therapeutic approach to bone loss.

ACKNOWLEDGEMENTS

The present study was supported by a grant from the Thailand Research Fund MRG4880111, Center for Excellence Research Fund, Chulalongkorn University and the Thailand Research Fund for Senior Research Scholar. The authors would like to express their gratitude to the staff of the Center of Excellence in Viral Hepatitis Research, Chulalongkorn University and Chulalongkorn Memorial Hospital for their efforts in this study. We also would like to thank the Venerable Dr. Mettanando Bhikkhu and Ms. P. Hirsch for reviewing the manuscript and Mr. Wasan Punyasang for statistical assistance.

References

1. Lefkowitch JH. Biliary atresia. *Mayo Clin Proc* 1998; 73: 90–5.
2. Karrer FM, Price MR, Bensard DD, Sokol RJ, Narkewicz MR, Smith DJ, et al. Long-term results with the Kasai operation for biliary atresia. *Arch Surg* 1996; 131: 493–6.
3. Heubi JE, Tsang RC, Steichen JJ, Chan GM, Chen IW, DeLuca HF. 1,25-Dihydroxyvitamin D3 in childhood hepatic osteodystrophy. *J Pediatr* 1979; 94: 977–82.
4. D'Antiga L, Moniz C, Buxton-Thomas M, Cheeseman P, Gray B, Abraha H, et al. Bone mineral density and height gain in children with chronic cholestatic liver disease undergoing transplantation. *Transplantation* 2002; 73: 1788–93.
5. Argao EA, Specker BL, Heubi JE. Bone mineral content in infants and children with chronic cholestatic liver disease. *Pediatrics* 1993; 91: 1151–4.
6. Janes CH, Dickson ER, Okazaki R, Bonde S, McDonagh AF, Riggs BL. Role of hyperbilirubinemia in the impairment of osteoblast proliferation associated with cholestatic jaundice. *J Clin Invest* 1995; 95: 2581–6.
7. Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM. Positional cloning of the mouse obese gene and its human homologue. *Nature* 1994; 372: 425–32.
8. Sørensen TI, Echwald S, Holm JC. Leptin in obesity. *BMJ* 1996; 313: 953–4.
9. Friedman JM, Halaas JL. Leptin and the regulation of body weight in mammals. *Nature* 1998; 395: 763–70.
10. Huang L, Li C. Leptin: a multifunctional hormone. *Cell Res* 2000; 10: 81–92.
11. Rosen HN, Moses AC, Garber J, Iloputaife ID, Ross DS, Lee SL, et al. Serum CTX: a new marker of bone resorption that shows treatment effect more often than other markers because of low coefficient of variability and large changes with bisphosphonate therapy. *Calcif Tissue Int* 2000; 66: 100–3.
12. Brown JP, Delmas PD, Malaval L, Edouard C, Chapuy MC, Meunier PJ. Serum bone Gla-protein: a specific marker for bone formation in postmenopausal osteoporosis. *Lancet* 1984; 1: 1091–3.
13. Bachrach LK, Hastie T, Wang MC, Narasimhan B, Marcus R. Bone mineral acquisition in healthy Asian, Hispanic, black, and Caucasian youth: a longitudinal study. *J Clin Endocrinol Metab* 1999; 84: 4702–12.
14. Feranchak AP, Ramirez RO, Sokol RJ. Medical and nutritional management of cholestasis. In: Suchy RJ, Sokol RJ, Balistreri WF, eds. Liver Disease in Children, 2nd ed. Philadelphia, PA: Lippincott Williams & Wilkins; 2001: 195–235.
15. Chongsrisawat V, Ruttanamongkol P, Chaiwatanarat T, Chandrakamol B, Poovorawan Y. Bone density and 25-hydroxy vitamin D level in extrahepatic biliary atresia. *Pediatr Surg Int* 2001; 17: 604–8.
16. Kose M, Ozdemir MA, Gumus H, Karakukcu M, Akcakus M. Serum leptin levels in patients with childhood immune thrombocytopenic purpura. *J Pediatr Hematol Oncol* 2007; 29: 23–6.
17. Aliyazicioglu Y, Ozkaya O, Yakut H, Islek I, Alvur M. Leptin levels in Henoch- Schonlein purpura. *Clin Rheumatol* 2007; 26: 371–5.
18. Ertekin V, Orbak Z, Selimoglu MA, Yildiz L. Serum leptin levels in childhood celiac disease. *J Clin Gastroenterol* 2006 40: 906–9.
19. Ahmed ML, Ong KK, Thomson AH, Dunger DB. Reduced gains in fat and fat-free mass, and elevated leptin levels in children and adolescents with cystic fibrosis. *Acta Paediatr* 2004; 93: 1185–91
20. Iorio R, Sepe A, Giannattasio A, Cirillo F, Spagnuolo MI, Franzese A, et al. Immune phenotype and serum leptin in children with obesity-related liver disease. *J Clin Endocrinol Metab* 2006; 91: 341–4.
21. Selimoglu MA, Ertekin V. Is leptin a predictive factor in the end of therapy response in chronic hepatitis B? *Pediatr Int* 2005; 47: 378–81.
22. Roberts GA, Holt RI, Ghatei MA, Baker AJ, Bloom SR, Miell JP. Serum leptin and insulin in paediatric end-stage liver disease and following successful orthotopic liver transplantation. *Clin Endocrinol (Oxf)* 1998; 48: 401–6.
23. Onodera K, Kato A, Suzuki K. Serum leptin concentrations in liver cirrhosis: relationship to the severity of liver dysfunction and their characteristic diurnal profiles. *Hepatol Res* 2001; 21: 205–12.
24. Testa R, Franceschini R, Giannini E, Cataldi A, Botta F, Fasoli A, et al. Serum leptin levels in patients with viral chronic hepatitis or liver cirrhosis. *J Hepatol* 2000; 33: 33–7.
25. Greco AV, Mingrone G, Favuzzi A, Capristo E, Gniuli D, Addolorato G, et al. Serum leptin levels in post-hepatitis liver cirrhosis. *J Hepatol* 2000; 33: 38–42.
26. Roberts GA, Holt RI, Ghatei MA, Baker AJ, Bloom SR, Miell JP. Serum leptin and insulin in paediatric end-stage liver disease and following successful orthotopic liver transplantation. *Clin Endocrinol (Oxf)* 1998; 48: 401–6.
27. Mccullough AJ, Bugianesi E, Marchesini G, Kalhan SC. Gender-dependent alterations in serum leptin in alcoholic cirrhosis. *Gastroenterology* 1998; 115: 947–53.
28. Shimizu H, Kakizaki S, Tsuchiya T, Nagamine T, Takagi H, Takayama H, et al. An increase of circulating leptin in patients with liver cirrhosis. *Int J Obes Relat Metab Disord* 1998; 22: 1234–8.
29. Henriksen JH, Holst JJ, Moller S, Brinch K, Bendtsen F. Increased circulating leptin in alcoholic cirrhosis: relation to release and disposal. *Hepatology* 1999; 26: 1818–24.

30. Miell JP, Englaro P, Blum WF. Dexamethasone induces an acute and sustained rise in circulating leptin levels in normal human subjects. *Horm Metab Res* 1996; 28: 704–7.
31. Carrié Fässler AL, Bonjour JP. Osteoporosis as a pediatric problem. *Pediatr Clin North Am* 1995; 42: 811–24.
32. Kreipe RE. Bone mineral density in adolescents. *Pediatr Ann* 1995; 24: 308–15.
33. Jilka RL. Biology of the basic multicellular unit and the pathophysiology of osteoporosis. *Med Pediatr Oncol* 2003; 41: 182–5.